THE UNITED STATES PATENT AND TRADEMARK OFFICE	*Application Number*	New Application Div. of 08/981,340
	Filing Date	Herewith
	First Named Inventor	Gijsbertus F.M. VERHEIJDEN
	Group Art Unit	Unassigned
	Examiner Name	Unassigned
	Attorney Docket Number	2355-133
Title of the Invention:	NOVEL PEPTIDES FOR USE IN TREATMENT OF T-CELL MEDIATED CARTILAGE DESTRUCTION IN AUTOIMMUNE DISEASES	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination on the merits, please amend the above-referenced application as follows:

IN THE SPECIFICATION:

Page 1, at line 5, insert the title, --Field of the Invention--; and

Page 1, at line 16, insert the title, --Background of the Invention--.

Page 3, at line 11, insert the title, --Summary of the Invention--.

Page 3, between lines 19 and 20, insert the title --Detailed Description of the Invention--.

Please delete pages 14-16 and renumber pages 17-18 as 14-15.

Please enter the amendments to the specification as found in the attached sheets. The pertinent substitute paragraphs of the specification are found in the attached sheets.

IN THE ABSTRACT:

Please enter the Abstract of the Disclosure submitted herewith on a separate sheet following the amended claims.

SEQUENCE LISTING:

Please replace the sequence listing on pages 14-16 with the attached sequence listing.

IN THE CLAIMS:

Please cancel claims 1-10.

Please add new claims 11, 12, 13, 14, 15 and 16 as shown on the following pages.

Marked-up sheets of the amended specification paragraphs are attached to this Amendment. Inserted material is underlined and deleted material is enclosed within brackets.

New Paragraph for First Line of Specification

CROSS-REFERENCE TO RELATED APPLICATION

The present application is a divisional application of Serial No. 08/981,340, filed 18 December 1997, which is a U.S. National Stage filing under 35 U.S.C. §371 of PCT/EP96/02605, which claims foreign priority to EP 952016566 filed on 19 June 1995.

Clean Copy of Amended Specification - paragraph at page 4, line 32 - page 5, line 10

Although articular cartilage proteins are considered to be the target autoantigens competent of stimulating autoaggressive T cells involved in the destruction of articular cartilage in autoimmune diseases, it was not until the present invention that these MHC Class II binding T-cell epitopes associated with cartilage-responsive autoreactive T cells have been identified on the cartilage proteins, in particular on HAG and HCLP. The peptides according to the invention resemble these MHC Class II binding T-cell epitopes, thus providing T-cell reactive peptides which can be used in the peptide-induced T cell tolerance therapy. Accordingly, patients can be treated with the peptides according to the invention to induce specific T-cell tolerance not only to the administered peptides but to the target autoantigens HAG and HCLP as well. As other components of the immune system are not affected by the peptides according to the invention, the immune system of the patient will remain intact and will be able to protect the patient against other infections.

Clean Copy of Amended Specification - paragraph at page 5, lines 15-32

Peptides according to the invention have been described. Perin et al., FEBS Letters 206:73 (1986) describes the structural relationship between link proteins and proteoglycan monomers and discloses a peptide fragment obtained after tryptic digestion of the link protein. The peptide fragment has the amino acid sequence SSAGWLADRSVRYPISKARPNXGG. Goetinck et al., J. Cell Biol. 105:2403-2408 (1987) discloses the peptides NAGWLSDGSVQYPITKPREP and DAGWLADGSVRYPISRPRKR which correspond to the amino acid residues Asn^{207}- Pro^{226} and Asp^{306}-Arg^{325} respectively of the primary structure of link protein. Said peptides were synthesized

to study the interactions between link protein and hyaluronic acid and said amino acid residues were found to be involved in the binding of link protein to hyaluronic acid. Neame et al., J. Biol. Chem. 261(8):3519-3535, (1986) describes the elucidation of the primary structure of link protein from rat chondrosarcoma proteglycan aggragate. Analysis of a triptic digest of the link protein revealed a fragment having the amino acid sequence GGLDWCNAGWLSDGSVQYPITKPR. Perides et al., J. Biol. Chem. Vol. 264, no. 10:5981-5987 (1989) describes the isolation and partial characterization of a glial hyaluronate-binding protein (GHBP). Tryptic digestion of GHBP results in several peptide fragments, one of which having the amino acid sequence EQLFAAYEDGFEQCDAGWLADQTVRYPIRAPRVGCY.

Clean Copy of Amended Specification - paragraph at page 6, lines 14-22

The peptides according to the invention can also be prepared by recombinant DNA techniques. A nucleic acid sequence coding for a peptide according to the invention or a multimer of said peptides is inserted into an expression vector. Suitable expression vectors are, amongst others, plasmids, cosmids, viruses and YAC's (Yeast Artificial Chromosomes) which comprise the necessary control regions for replication and expression. The expression vector can be brought to expression in a host cell. Suitable host cells are, for instance, bacteria, yeast cells, and mammalian cells. Such techniques are well known in the art, see for instance Sambrooke et al., Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989.

Clean Copy of Amended Specification - paragraph at page 6, lines 24 to page 7, line 2

According to the invention, patients suffering from T-cell mediated destruction of the articular cartilage can be treated with a therapeutical composition [comprizing] <u>comprising</u> one or more peptides according to the invention and a pharmaceutical acceptable carrier. Administration of the pharmaceutical composition according to the invention will induce tolerance of the specific autoreactive T cells of these patients to the autoantigenic proteins in the articular cartilage under attack and other self antigens which display the identified MHC Class II binding T cell epitopes

characterized by one of the amino acid sequences of SEQ ID NO:1-6. More specifically, administration of the pharmaceutical composition according to the invention will induce tolerance of the specific autoaggressive T cells to the autoantigens HAG [end] and HCLP. The induced tolerance thus will lead to a reduction of the local inflammatory response in the articular cartilage under attack.

Clean Copy of Amended Specification - paragraph at page 12, lines 1-14

PBMC obtained from heparinized venous peripheral blood were isolated by standard centrifugation on a Ficoll-Paque gradient. Cells were cultured in three- or four-fold at a concentration of 1,5 x 10^5 cells/well in DMEM/Ham's F12 medium supplemented with 10% heat-inactivated pooled human serum, L-glutamine, 2-ME and antibiotics in flatbottomed microtiter plates. Cells were incubated in medium alone or in the presence of PHA (2.5 µg/ml) or in the presence of antigens, including the chicken proteoglycan fraction, the chicken collagen fraction, sonicated *Mycobacteriium tuberculosis* or the peptides HAG1, HAG2, HAG3 and HCLP1 in concentrations of 50 µg/ml, 5 µg/ml or 0.5 µg/ml. Cultures were incubated in a total volume of 210 µl for 4, 5, 6 or 7 days at 37°C in a humidified atmosphere of 5% CO_2. Cultures were pulsed with 0.5 µCi (1.85 x 10^4 Bq) [^{3}H]Thymidine ([^{3}H]TdR) for the last 18 hours of cell culture. Cells were harvested on glassfibre filters and [^{3}H]TdR incorporation was measured by gasscintilation. Note that counting by gasscintilation is fivefold less efficient compared to liquid scintilation. Therefore, filters were measured for 5 min (Packard Matrix 96 β-counter, Meriden CT).

Newly Added Claims 11-16

11. (New) A method for treating autoimmune diseases comprising administering a tolerance inductive amount of a peptide selected from the group consisting of:

(a) a peptide having 13-55 amino acid residues, said peptide comprising the amino acid sequences $AGWLX_1DX_2X_3X_4X_5YPI$ (SEQ ID NO:1) in which X_1 is A or S, X_2 is Q,R, or G, X_3 is T or S, X_4 is V or L and X_5 is R or Q;

(b) a peptide AGWLADQTVRYPI (SEQ ID NO:3);

(c) a peptide AGWLADRSVRYPI (SEQ ID NO:4);

(d) a peptide AGWLADGSLRYPI (SEQ ID NO:5);

(e) a peptide AGWLSDGSVQYPI (SEQ ID NO:6).

12. (New) The method of claim 11, wherein said peptide is a peptide having 13-55 amino acid residues, said peptide comprising the amino acid sequences $AGWLX_1DX_2X_3X_4X_5YPI$ (SEQ ID NO:1) in which X_1 is A or S, X_2 is Q,R, or G, X_3 is T or S, X_4 is V or L and X_5 is R or Q.

13. (New) The method claim 11, wherein said peptide is a peptide AGWLADQTVRYPI (SEQ ID NO:3).

14. (New) The method claim 11, wherein said peptide is a peptide AGWLADRSVRYPI (SEQ ID NO:4).

15. (New) The method claim 11, wherein said peptide is a peptide AGWLADGSLRYPI (SEQ ID NO:5).

16. (New) The method claim 11, wherein said peptide is a peptide AGWLSDGSVQYPI (SEQ ID NO:6).

Newly Added Abstract

ABSTRACT

The invention relates to the use of novel peptides in a peptide-induced tolerance therapy for the induction of tolerance to autoaggressive T cells associated with T-cell mediated articular cartilage destruction in autoimmune diseases, more specifically arthritis. The invention furthermore embraces pharmaceutical compositions comprising said peptides and a diagnostic method for the detection of autoreactive T cells in a test sample, said T cells being associated with T-cell mediated articular cartilage destruction in autoimmune diseases and test kits to be used in said method.

REMARKS

The specification has been amended to conform with amendments made in the parent application. Claims 11-16 have been added to claim a method for treating an autoimmune disease which was correspond to claim 11 of the parent application which was subject to a restriction requirement.

The SEQ ID NO:10 on page 5, line 21 is being corrected to conform to Goetinks et al. from which the sequence was taken.

The substitute Sequence Listing is identical to that filed on March 10, 2000 by certified mail in parent Application Serial No. 08/981,340. The substitute Sequence Listing does not contain any new matter and its entry is requested.

RESPECTFULLY SUBMITTED,					
NAME AND REG. NUMBER	Jeffrey L. Ihnen, Reg. No. 28,957				
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City	Washington	*State*	D.C.	*Zip Code*	20004
Country	U.S.A.	*Telephone*	202-783-6040	*Fax*	202-783-6031

Attachment: Marked-up copy of amendments.

Marked-Up Copy of Amended Specification - paragraph at page 4, line 32 - page 5, line 10

Although articular cartilage proteins are considered to be the target autoantigens competent of stimulating autoaggressive T cells involved in the destruction of articular cartilage in autoimmune diseases, it was not [untill] until the present invention that these MHC Class II binding T-cell epitopes associated with cartilage-responsive autoreactive T cells have been identified on the cartilage proteins, in particular on HAG and HCLP. The peptides according to the invention resemble these MHC Class II binding T-cell epitopes, thus providing T-cell reactive peptides which can be used in the peptide-induced T cell tolerance therapy. Accordingly, patients can be treated with the peptides according to the invention to induce specific T-cell tolerance not only to the administered peptides but to the target autoantigens HAG and HCLP as [wel] well. As other components of the immune system are not affected by the peptides according to the invention, the immune system of the patient will remain intact and will be able to protect the patient against other infections.

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Marked-Up Copy of Amended Specification - paragraph at page 6, lines 24 to page 7, line 2

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:)
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Gijsabertus F.M. VERHEIJDEN et al)
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Serial No.: New- Div. of 08/981,340)
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Filed: Herewith)
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For: NOVEL PEPTIDES FOR USE IN TREAT-)
MENT OF T-CELL MEDIATED CARTILAGE
DESTRUCTION IN AUTOIMMUNE DISEASES

LETTER PURSUANT TO 37 CFR 1.821(e)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In the matter of the above-identified divisional application, filed concurrently herewith, Applicants note that the computer readable form of the substitute Sequence Listing of the present application is identical to the computer readable form submitted in parent application Serial No. 08/981,340 on 18 December 1997. In accordance with 37 CFR 1.821(e), please use this third-filed computer readable form filed in that application as the computer readable form for the present application.

It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the present application.

Respectfully submitted,

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